REMARKS

This is in response to the office action dated December 11, 2003. After entry of the present amendment, claims 31 and 33-64 are pending. The Examiner has withdrawn process claims 49-57 and 60 from Examination under 37 C.F.R. 1.142(b), but acknowledges that if a product claim is found allowable, withdrawn process claims depending from or otherwise including all of the limitations of the allowable product claim will be rejoined in accordance with MPEP §821.04. With this response, claims 33, 35-40, 42-43, and 45-57 have been amended. New claims 61 to 66 have been added. No new matter has been added with this response.

Claims 31, 33, 35, 37, 39-40, 42-43, 45-57, and 59 have been amended in matters of formal claim language.

Claim 33 has also been amended to recite a plasmid vector comprising a transcription control sequence. This is supported by the specification at, *e.g.*, page 18, first paragraph; pages 18-19, bridging paragraph; and page 19, last paragraph.

Claims 36 and 38 have been amended to recite specific *udp* and *deoD* sequences. This is supported, *e.g.*, by the descriptions in the sequence listing filed on June 25, 2001 of SEQ ID NO:6, where it is stated that residues 243 to 1021 correspond to the *udp* gene and that residues 1037 to 1766 correspond to the *deoD* gene.

Claim 47 has also been amended to be directed to prokaryotic host cells. This amendment is supported by the specification at, *e.g.*, page 6, last full paragraph.

Claim 48 has also been amended to recite the step of culturing host cells containing a recombinant plasmid expression vector according to claim 31. This is supported by, e.g., Examples 5 and 6, pages 21-24 of the specification.

New claims 61-66 are supported by the specification at, *e.g.*, page 10, last paragraph, page 12, 1st paragraph, and pages 5-6, bridging paragraph.

Each of the Examiner's objections and rejections are discussed below.

Priority

The Examiner states that the foreign priority document has not been filed in the instant application. A copy of priority application MI 98 A 002792, filed December 23, 1998, will follow.

Oath/Declaration

The Examiner contends that the Declaration is defective because it does not recite the city and either state or foreign country of residence of each inventor. It is respectfully submitted that the Declaration filed on June 25, 2001 properly identifies the city and foreign country of each inventor. See, pages 2-3 of the Declaration (see copy enclosed with this response). This objection should therefore be withdrawn.

Sequence Compliance

The Examiner refers to sections in the specification where there are nucleotide or amino acid sequence not identified by a sequence identifier. With this response, and as shown in the section entitled "Amendments to the Specification", these sections have been amended to properly recite sequence identifiers.

In addition, accompanying this submission is a substitute sequence listing, in the form of both a paper copy and diskette, where nucleotide or amino acid sequences not previously identified by a sequence identifier. In the section entitled "Amendments to the Specification", the specification has been amended to replace the sequence listing filed on October 9, 2001, with the substitute sequence listing.

Title of the Invention

The Examiner suggests replacing the title of the invention. Pursuant to the Examiner's suggestion, and as shown in the section entitled "Amendments to the Specification", the title of the

invention is now "Vectors, Host Cells, and methods for Production of Uridine Phosphorylase and Purine Nucleotide Phosphorylase".

Brief Description of the Drawings

The Examiner notes that there is no section in the specification entitled "Brief description of the Drawings". As shown in the section entitled "Amendments to the Specification", such a section has now been added to the specification. The description of each drawing is identical to the figure text of the original drawings filed in the parent application of this application; PCT application PCT/EP99/10416, published as WO 00/39307. Per the preliminary amendment filed with the instant application (see June 25, 2001 submission entitled "PCT Continuing Application"), this PCT application is incorporated by reference in its entirety into the present specification.

Claim Objections

The Examiner has objected to claims 39 and 40 for not identifying pBR322 and pET29c as plasmids. With this response, claims 39 and 40 have been amended to recite "plasmid pBR322" and "plasmid pET29c", respectively.

The Examiner has also objected to claim 43 for not using a proper sequence identifier. With this response, claim 43 has been amended to recite proper sequence identifiers.

Utility Rejection

The Examiner has rejected claim 48 for reciting a use without setting forth any steps involved in the process. With this response, claim 48 has been amended to recite a step of culturing host cells containing a recombinant plasmid expression vector. It is thereby believed that this rejection has been overcome and should be withdrawn.

Indefiniteness Rejections

The Examiner has rejected claim 33 as allegedly indefinite for not reciting a transcription control sequence. With this response, claim 33 has been amended to recite a transcription control sequence.

The Examiner has also rejected claims 35 and 37 as allegedly indefinite in the recitation of "sequence *udp*" and "sequence *deoD*". With this response, claims 35 and 37 have been amended to recite "an *udp* gene" and "a *deoD* gene", respectively.

The Examiner has also rejected claims 36 and 38 as allegedly indefinite for reciting EMBL database sequences. With this response, claims 36 and 38 call for specific residues of SEQ ID NO:6.

The Examiner has also rejected claim 42 as allegedly indefinite for reciting "aminoacid units". With this response, claim 42 has been amended to recite "a polypeptide linker of more than one amino acid".

The Examiner has rejected claims 45-46 as allegedly indefinite for reciting the phrase "preferably", and claim 46 is also cited as "confusing". With this response, claims 45-46 no longer recite the term "preferably", and claim 46 recites *E. coli* strain K12 or B.

Claim 47 has been rejected as allegedly indefinite because of alleged unclarity in the recitation of the expression vector. With this response, claim 47 has been amended to recite prokaryotic host cells containing a plasmid vector according to claim 41.

Claim 48 has been rejected as allegedly indefinite because it does not recite a method step. With this response, claim 48 recites a step of culturing host cells containing a recombinant plasmid expression vector according to claim 31.

Written Description

The Examiner has rejected claims 31, 33-42, 44-48, and 58-59 as allegedly not complying with the written description requirement. Specifically, the Examiner contends that the specification "merely describes the functional features of the genus [of genes] without providing any definition of the structural features of the species within the genus" (office action, page 7, last paragraph).

In contrast to the Examiner's reasoning, the claims fully comply with the written description requirement, as the claims are directed to a novel plasmid comprising sequences that were well-known in the art at the time of filing of the application. Thus, the claims are not drawn to a novel genus of genes encoding polypeptides having UDP or PNP activity. For example, the *udp* and *deoD* sequences from *E. coli*, recited in claims 36 and 38, have been known and publicly available since at least 1991, as shown in the EMBL database entries attached as Exhibits 1 and 2, respectively. The sequences referred to as AC CG01747 (udp) and AC CG00327 (deoD) in the specification on page 5, 1st full paragraph, were also described in publications published 1991 or earlier, as shown in the database excerpts attached hereto as Exhibits C and D, CG01747 and CG00327, respectively. Additionally, *upd* and *deoD* sequences from *Klebsiella sp.* LF 1202 are described in a 1995 publication by Takehara et al. (6/25/2001 IDS, ref. No. 10); and *E. coli udp* mutants were evaluated prior to 1991 as reported by Hershfield et al. (6/25/2001 IDS, ref. No. 7).

Thus, given that *udp* and *deoD* sequences from mesophilic bacteria were known, used, and even manipulated long before the filing of the present application, the skilled artisan would have no doubt that the applicants were in possession of the full scope of the claimed invention at the time the application was filed. Particularly, as set forth by the MPEP, section 2163.II.A.2,

> "Information that is well known in the art need not be described in detail in the specification. See, *e.g., Hybritech, Inc. v. Monoclonal Antibodies, Inc.* 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986).

It is further noted that claim 34 recites that the mesophilic bacterium is *E.coli*. Given the well-characterized genome and biology of this organism, the skilled artisan could easily have identified, characterized, and used an *udp* and/or *deoD* sequence from this organism.

Accordingly, for all of the above reasons, it is submitted that all of the claims, including new claims 61-66 (which depend from claim 33 or otherwise have the same limitations as claim 33 with respect to the sequences encoding uridine phosphorylase or purine nucleoside phosphorylase activity) comply with the written description requirement. Reconsideration and withdrawal of this rejection is therefore respectfully requested.

Enablement

The Examiner has rejected claims 31, 33-42, 44-48, and 58-59 as allegedly not enabled by the specification. Specifically, while the office action acknowledges that the specification enables an expression vector comprising nucleotides 243-1021 of SEQ ID NO:1 and 231-960 of SEQ ID NO:3 (page 9, 1st paragraph), the Examiner contends that the claims are overly broad because they encompass all recombinant vector comprising any gene encoding a mesophilic uridine phosphorylase enzyme and any gene encoding a mesophilic bacterial purine nucleoside phosphorylase. The Examiner also cites the *In re Wands* factors, arguing that the claims encompass mutant *udp* and *deoD* sequences, and that the predictability of which changes can be tolerated in an encoded protein's amino acid sequence and obtain the desired activity is low.

It is respectfully submitted that the claims comply with the written description requirement. First, the claims call for "at least one gene sequence of a mesophilic bacterium coding for a polypeptide having uridine phosphorylase enzyme activity and at least one gene sequence of a mesophilic bacterium coding for a polypeptide having purine nucleoside phosphorylase enzyme activity", thus reciting that the sources of the respective sequences are mesophilic bacteria. Secondly, because *udp* and *deoD* genes have long been known in the art, as outlined above, and since assays for testing for uridine phosphorylase enzyme activity and purine nucleoside phosphorylase enzyme were likewise well-known at the time the present application was filed, the

skilled artisan would have had to engage in no more than routine experimentation to identify whether a particular mesophilic bacteria possessed such activity and thereafter retrieve the *udp* and *deoD* sequences. Whether a bacteria is a mesophilia or not would probably be one of the most basic and straightforward microbiology test since one simply assays for the temperature where optimum growth occurs (mesophilic bacteria have growth optimum at temperatures between 30 and 37°C; see specification at page 5, 1st full paragraph). Subsequent testing for uridine phosphorylase and purine nucleoside phosphorylase activity could be carried out by, *e.g.*, by any of the assays described in the Hershfield reference (*supra*) or, indeed, the present specification (see, e.g., Example 7, page 24 *et seq.*). Additionally, a detailed description of the retrieval of *udp* and *deoD* sequences from the bacterial strain *Klebsiella sp.* LF 1202 is provided by the Takehara et al. 1995 reference cited above.

Accordingly, the methods to test for mesophilic bacteria, uridine phosphorylase activity, and purine nucleoside phosphorylase activity, and methods to retrieve the corresponding gene sequences, were established and routine at the time the present application was filed. Notably, this issue is directly analogous to the one faced by the Federal Circuit in *In re Wands* (858 F.2d 731, 8 USPQ2d 1400 (Fed. Cir. 1988)). In this case, the PTO was found to have erred in rejecting the applicant's claim to immunoassay methods using a specified generic class of antibodies (IgM). The applicant made a public deposit of a hybridoma cell line that secreted only a specific antibody, but disclosed methods of producing and screening other hybridomas for the desired antibody specificity and class. As summarized by the court (*Id.*):

> Enablement is not precluded by the necessity for some experimentation such as routine screening of hybridoma cells that secrete a desired monoclonal antibody from other cells derived from an immunized animal.

In the *In re Wands* case, the applicants could hardly predict what amino acid sequences would bind to the desired antigen, but performed routine screening to identify antibodies that had the sought-after specificity or "activity". This is, again, analogous to the instant claims, where mesophilic bacteria having uridine phosphorylase and/or purine nucleoside phosphorylase activity can be identified, by routine screening, without prior knowledge as to which positions of the corresponding gene sequences might or might not lead to retained or improved activity or stability.

Finally, it is noted that after entry of the present amendment, claim 34 calls for the mesophilic bacterium being *E. coli*, and claims 35 and 37 call for *udp* and *deoD* genes, which genes have been known since at least 1991, and that claim 36 and 38 recite specific *udp* or *deoD* sequences. In this context, it is noted that the Examiner's concerns about the predictability of sequence modifications are alleviated by the Hershfield reference (supra), which describes not only amino acid substitutions but also extensive modifications to the *E. coli* PNP enzyme by attaching polyethylene glycol to the modified residues, and notes that "[t]he wild-type and RK3 enzymes had similar catalytic activity, antigenicity, and immunogenicity" (see Hershfield abstract). Moreover, the Hershfield reference selects residues for modification by inspection of the wild-type *deoD* sequence and by computer-assisted analysis of PNP secondary structure, thus showing that simple and well-established techniques can be used to avoid or reduce the alleged "unpredictability."

For all of the above reasons, it is submitted that all of the claims, including new claims 61-66 (which depend from claim 33 or otherwise have the same limitations as claim 33 with respect to the sequences encoding uridine phosphorylase or purine nucleoside phosphorylase activity) are enabled by the specification, in view of the level of skill in the art at the time of filing of the application. Reconsideration and withdrawal of this rejection is therefore respectfully requested.

Obviousness

Claims 31, 34-38, 40-42, 44-48, and 58-59 have been rejected as allegedly obvious over Krenitsky et al. (U.S. 4,347,315) in view of Walton et al. (Nucleic Acid Res 17:6741), Hershfield et al. (6/25/2001 IDS, ref. No. 7), Bulow et al. (Trends Biotech 9:226-231) and the Novagen 1997 catalog. Specifically, the Examiner argues that the Krenitsky patent describes a method for preparing imidizo(4,5-c)pyridine derivatives in aqueous suspension comprising *E. coli* PNP and UdP; that the Walton and Hershfield references teach *upd* and *deoD* sequences, respectively; that the Bulow reference teaches fusion enzymes; and that the Novagen catalog describes plasmid pET29c with kanamycin resistance and the K12 strain; and that it would have been *prima facie* obvious to combine these references to arrive at the present invention.

Claims 31, 34-39, 41-42, 44-45, and 47-48 have also been rejected as allegedly obvious over the Krenitsky patent in view of the Walton, Hershfield, and Bulow references as well as Sambrook et al. ("Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Press 1989). Specifically, the Examiner teaches that the Krenitsky patent and the Walton, Hershfield, and Bulow references teach as above, that Sambrook teaches plasmid markers (including tetracyclin resistance) for selecting clones comprising a plasmid; and that it would have been *prima facie* obvious to combine these references to arrive at the present invention.

In contrast to the Examiner's reasoning, the claims are unobvious over both combinations of references cited in the office action. The main reasons for the failure of these reference combinations to render the claims obvious are that (1) the primary reference, the Krenitsky patent, teaches away from the present invention; and (2) the combinations of references fail to provide a reasonable expectation of success.

- ***The Krenitsky Patent Teaches Away From the Claimed Invention***

This patent is directed to a method of preparing 4-substituted-1-B-D-ribofuranosyl-1H-imidazo(4,5-c)pyridines by reacting a pyridine precursor with a ribose donor system comprising ribose-1-phosphate and a phosphorylase type enzyme (col. 2, lines 42-47). This can apparently be done in a two-step "one-pot" process, *i.e.*, an *in vitro* suspension/solution, where ribose-1-phosphate produced by, *e.g.*, a pyrimidine nucleoside phosphorylase reaction, is converted to the product by using a purine nucleoside phosphorylase (col. 3, line 23 to col. 4, line 13). The patent also calls *B. stearmophilus* and *E. coli* B "excellent sources" of such enzymes (col. 4, lines 14-29).

Importantly, however, the subsequent sections of the Krenitsky patent teaches that "crude", *i.e.*, unpurified enzyme systems suffer from disadvantages such as a decreased product yield (col. 4, lines 30-43):

> It has been found that crude enzyme preparations are less suitable than purified preparations. This is due to the fact that crude preparations contain troublesome nucleic acids as well as enzymes other than those required for the process of the present invention. The extraneous enzymes in crude

> preparations catalyse undesirable alterations of substrates and products, and may even cause proteolysis of the required enzymes themselves. These factors decrease not only the yield of the desired products but also the ease with which they can be isolated from reaction mixtures.
>
> In most cases therefore, it is desirable to purify the crude enzyme preparations before addition to the reaction mixture.

Accordingly, the Krenitsky patent unequivocally teaches that for a high product yield, "crude" enzyme preparations must be purified so that the enzymes are removed from other components present in the environment in which they naturally occur, *i.e.*, a cell. This is in direct contrast to the present invention, where high yields of product can be achieved with the enzymes remaining in a natural environment, *i.e.*, a host cell. Notably, new claims 61 and 63 set forth particular embodiments where up to 1000 times higher product yield is obtained in a transformed as compared to a non-transformed host cell using the claimed plasmids.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). (MPEP 2143.01). In the instant case, when considering the Krenitsky patent as a whole, it cannot be combined with the other references, as suggested by the Examiner, to arrive at an expression plasmid for use in a cellular system.

- ***There is No Reasonable Expectation of Success***

As discussed above, because of its teachings as a whole, the Krenitsky patent cannot be combined with the other references. For this reason alone, the claims are unobvious over the combinations of references suggested by the Examiner. However, even when forcefully combining the Krenitsky patent with the references proposed by the Examiner, the resulting combination provides no reasonable expectation of success in arriving at the presently claimed invention.

The Krenitsky patent states that "[f]or the purposes of the present invention ... aerobic bacteria such as *B. stearothermophilus* and especially *E. coli* B ... were found to be excellent sources of such enzymes" (column 4, lines 18-23), thereby teaching that in an *in vitro* setting,

thermophilic (*B. stearothermophilus*) and mesophilic (*E. coli*) UdP and PNP enzymes are both effective. However, as reported in JP-06-253854 (ref. No. 4 in 6/25/2001 IDS (Abstract only); full translation in concurrently filed IDS), bacterial plasmids containing the gene sequences for UdP and PNP from thermophilic *B. stearmophilus* only yielded a 6-8-fold improvement of productivity as compared to one of the non-transformed control strain (see translation of JP-06-253854, page 31, last paragraph). These results are even more disappointing in view of the facts that

(a) an elevated temperature permits higher enzyme kinetics (see instant specification at page 4, 2nd full paragraph and page 12, 1st paragraph), and
(b) the very reason for using thermophilic enzymes in JP-06-25384 was to promote production yield over side reactions carried out by normal *E. coli* enzymes, almost all of which "are inactivated in the case of conducting the synthesizing reaction at a high temperature" (see translation of JP-06-253854, page 24, last paragraph).

Accordingly, since the Krenitsky patent teaches that thermophilic and mesophilic enzymes are both effective, and since JP-06-25384 essentially shows that thermophilic enzymes are not particularly effective, the skilled artisan would not have had any reasonable expectation that a plasmid expression vector based on mesophilic enzymes would have been successful or even worthwhile to pursue prior to the present invention. None of the other references cited by the Examiner would have given the skilled artisan a reasonable, or at least improved, expectation of success in obtaining a plasmid effective for conducting UDP and PNP reactions in a recombinant system, as the Hershfield reference tests PNP activity of mutants after *in vitro* PEG-ylation, thus conducting activity tests *in vitro* as well; and since all of the remaining references are silent as to any PNP or UDP activity testing in recombinant systems.

By contrast, using the present plasmid containing mesophilic UdP and PNP at more moderate temperatures, 30°C, resulted in up to 1040 and 200 times UdP and PNP activity, respectively, even at 30°C (specification at page 12, 1st paragraph). Additionally, particular embodiments where the UDP and PNP enzyme activities are 120-1000 times higher than control are described in the specification at page 10, last paragraph, and are now set forth in new claims 61 and 63.

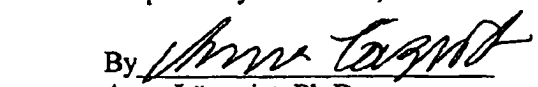
For all of the above reasons, it is respectfully submitted that all claims, including new claims 61-66, are unobvious over any combination of the references cited by the Examiner.

Conclusion

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue.

Dated: March 11, 2004

Respectfully submitted,

By 

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